Vol. 92, No. 2 April, 1947

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

EFFECTS OF HYDROGEN PEROXIDE PRODUCED IN THE MEDIUM BY RADIATION ON SPERMATOZOA OF ARBACIA PUNCTULATA

TITUS C. EVANS [1]

Radiological Research Laboratory, Columbia University, New York, N. Y., and Marine Biological Laboratory, Woods Hole, Massachusetts

In the course of a previous investigation (Evans et al., 1941, 1942a) it was found that roentgen radiation exerted effects on Arbacia sperm indirectly by producing a very temporary toxicity of the medium. The intermediary appeared to be the "activated water molecule," as postulated by Fricke (1935), and was characterized by the following phenomena. Sperm irradiated in sea water suffered immediate reduction in fertilizing capacity, whereas sperm irradiated "dry" were apparently unaffected in this respect. The smaller the concentration of sperm in the suspension during irradiation the greater the percentage made incapable of fertilization. Presence of proteins, such as egg albumin, afforded "protection," that is, greatly increased the percentage of sperm surviving irradiation. Unirradiated sperm did not suffer immediate reduction in fertilizing capacity when placed in water that had been exposed to amounts of radiation capable of producing such effects when the sperm were present during the irradiation. Under certain conditions "activated water" reacts to produce hydrogen peroxide (Risse, 1930; Taylor et al., 1933; and Fricke, 1934a) which is toxic to organisms and may account for some or all of the indirect effects of X-rays. To understand better the indirect action of X-rays on cells, comparative studies of the effects of irradiated water and hydrogen peroxide were made on Arbacia sperm and ova.

Materials and Methods

Male Arbacia were stimulated, by cutting through the oral region, to shed sperm in a dish of sea water. The suspension was centrifuged until the sperm were closely packed, then the supernatant fluid was removed. Before placing the sperm in test solutions, nine parts of sea water were mixed with one part of packed sperm in order to facilitate transfer of equal amounts of sperm suspension to the various dishes. One part of this suspension was added to ninety-nine parts of the test solution making a final concentration of 1 : 1,000 sperm. Percentage fertilizing capacity was determined by inseminating a given lot of freshly collected eggs with as many drops of control sperm as needed to produce 90–99 per cent of the eggs with

[1] It is a pleasure to express appreciation to Dr. G. Failla for his helpful criticisms and to Miss Anne R. Behan for technical assistance.

fertilization membranes. The same volume of sperm from the experimental medium was then tested with a lot of eggs similar to those used for the controls.

Samples of sperm from sea water and from experimental media were taken at regular intervals and tested for fertilizing capacity. The survival period was taken as the time required to reduce the percentage fertility of a given lot of sperm to less than 50 per cent. The actual survival time was somewhat longer in each case as some sperm would still be alive after the suspension had completely lost the ability to fertilize untreated eggs. A few observations were made of longevity as indicated by rate of oxygen consumption and motility of sperm. The relative decrease in survival time of different experimental lots was approximately the same whether these criteria or fertilizing capacity were employed. Sperm in a concentration of 1 : 10 suffered no change in fertilizing capacity during the time covered by these experiments. Therefore, when it became necessary to employ a new batch of eggs, the proper number of drops of sperm suspension to use in each test was determined by employing a freshly diluted 1 : 1,000 suspension. Cleavage time was taken as the number of minutes (at 25° C.) from the time of insemination until 50 per cent of the fertilized eggs had undergone division. Catalase solutions used were sea water dilutions of a stock extracted from sperm by distilled water. Albumin solutions were 0.1 gram per cent of powdered egg albumin made up in sea water and used immediately.

Fresh filtered sea water was irradiated in covered plastic dishes at 22–28° C. The material was cross-fired (from above and below) between two water-cooled X-ray tubes operated at 182 kv pk., and 25 ma (inherent filtration equivalent to approximately 0.2 mm. Cu). The intensity was usually 5,600 r per minute and the irradiation was given in one treatment.

Experimental Results

Effects of irradiated water

Results of exploratory experiments (Evans et al., 1942a; Evans, 1942) indicated that irradiated water reduced the survival time and prolonged the cleavage time of sperm placed therein. The amount of effect varied greatly from one lot of sperm to another. It appeared in general that irradiation below 50,000 r was ineffectual, around 100,000 r was definitely effective, and a dose of 224,000 r was near the optimum in affecting the water so that it reduced survival time of sperm. The delay in first cleavage (treated sperm + untreated ova) was noticeable when only low dosages (below 100,000 r) had been given to the water, but the effect did not appear to increase rapidly as the irradiation became greater. In the exploratory experiments chemical tests indicated presence of hydrogen peroxide in the heavily irradiated water, and this agent was also found to affect survival time and cleavage time of sperm. It was now of interest to determine quantitatively how much of the toxicity of the irradiated water should be attributed to hydrogen peroxide.

It was found that consistent results were obtained when sperm from several males were pooled so that successive experiments were made from the same original collection. The data are shown in Figure 1 and Figure 2. It may be seen in Figure 1 (top graph) that, in all four experiments, the water became more toxic as the amount of radiation was increased. Reduction in survival time by doses below

85,000 r was not very definite. The effect increased rapidly from 85,000 r to 140,000 r and increased, though less rapidly, from 140,000 r to 230,000 r. The latter finding may indicate that equilibrium was being reached between the rate of hydrogen peroxide formation and the rate of destruction by the radiation.

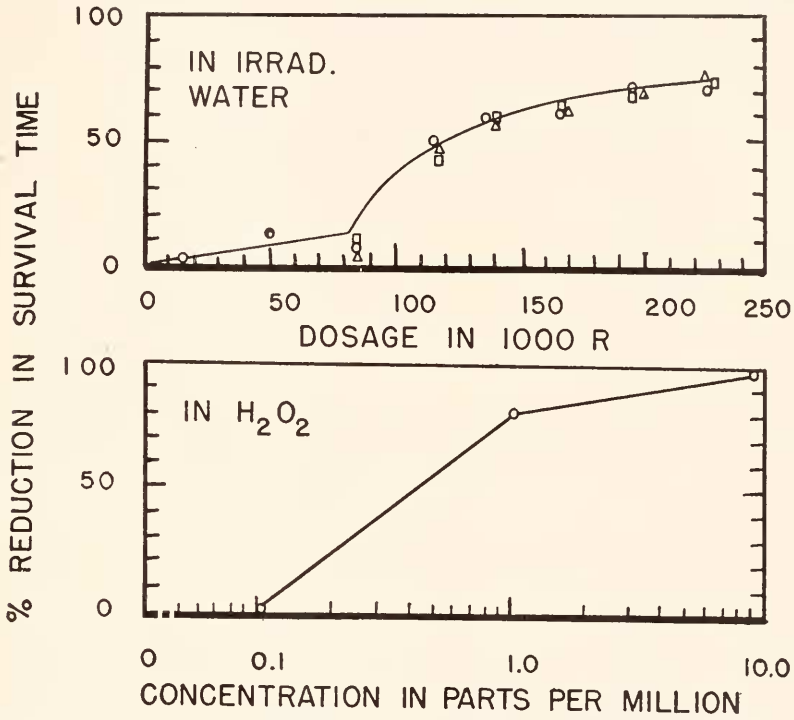

FIGURE 1. Upper graph: effect of different amounts of radiation on the toxicity of sea water to sperm. Toxicity is indicated by a reduction of the survival time (as measured by fertilizing capacity) of the sperm placed in the irradiated water as compared to the survival time of sperm in control sea water.

Lower graph: toxicity of different amounts of hydrogen peroxide in sea water to sperm placed therein.

The effect of retarding cleavage was greater as the irradiation of the water was increased (upper graph of Fig. 2). Also, the cleavage time was more delayed the longer the sperm had been in the irradiated water at the time of insemination. This is shown in the lower graph of Figure 2. The effect approached a maximum, at this concentration of the agent, in about an hour (at 25° C.).

Effects of hydrogen peroxide

Calculations based on data available in the literature (Fricke, 1934a, 1934b; Taylor, Thomas, and Brown, 1933) and color tests, using titanic chloride (Evans et al., 1942a), of the irradiated sea water indicated that we were dealing with concentrations of hydrogen peroxide in the neighborhood of 1 : 1,000,000. The effects of different concentrations of hydrogen peroxide [2] on the survival time are shown

[2] Merck's Superoxol reagent hydrogen peroxide (30 per cent).

in the lower graph of Figure 1. One would judge from a consideration of the two graphs of Figure 1 that the maximum amount of hydrogen peroxide produced in the irradiated water was approximately 1 : 1,000,000.

Hydrogen peroxide in a concentration of 1 : 1,000,000 retarded cleavage (lower graph of Fig. 2) and the increase in effect with longer exposures was very similar to that of sea water given an irradiation of 224,000 r.

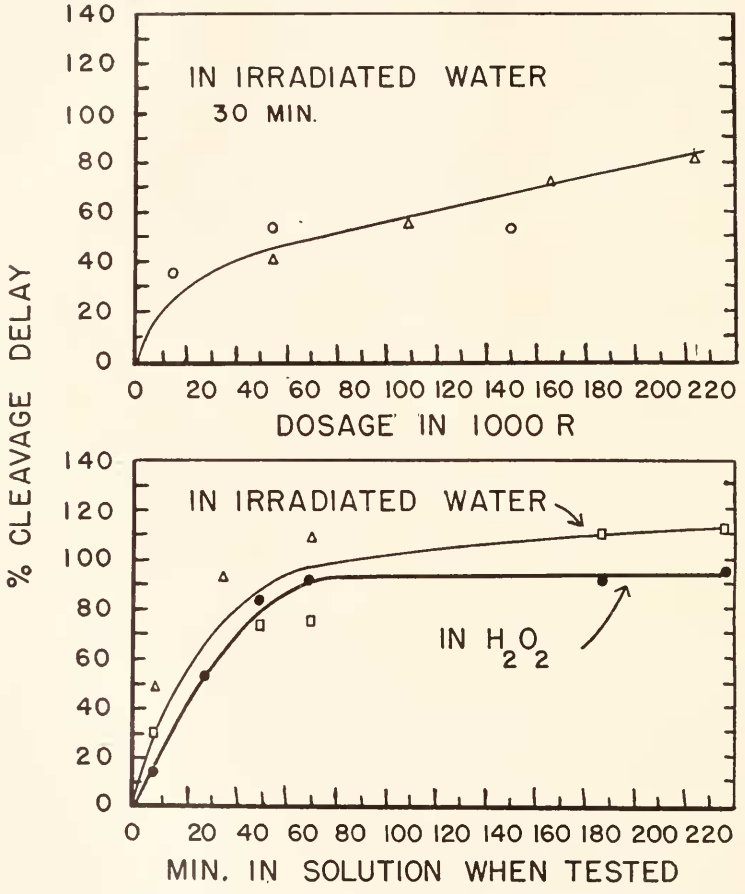

FIGURE 2. Upper graph: effect of different amounts of radiation on toxicity of sea water as indicated by a retardation in time of first cleavage. After sperm had been in the irradiated water for 30 minutes they were used to inseminate fertile eggs in fresh sea water. Cleavage delay is expressed as cleavage time over that of eggs inseminated with control sperm.

Lower graph: effects (on cleavage time) of exposing the sperm for different periods of time to irradiated water (224,000 r) and to water containing hydrogen peroxide (1 : 1,000,000).

Reduction in toxicity of irradiated water by catalase extract

't was found that a crude extract possessing catalase activity could be removed concentrated sperm suspensions.[3] This extract could be added to sperm in

[3] Report in preparation.

sea water without producing any apparent deleterious effects. Results of adding catalase to irradiated water are shown in Tables I and II. It will be seen that the catalase extract completely removed the toxic agent responsible for decrease in survival time (Table I) in all but three instances and greatly reduced the effect in these.

TABLE I

REDUCTION OF TOXICITY OF IRRADIATED WATER BY CATALASE EXTRACT

*(Percentage decrease in survival time as compared to sperm in control sea water)**

In irrad. water (160,000 r)	In irrad. water +inactive catalase**	In irrad. water +active catalase	In H_2O_2 (1:1,000,000)	In H_2O_2 +active catalase
50	50	8	—	—
92	—	0	90	0
55	55	0	—	—
38	38	0	—	—
81	43	22	—	—
83	48	0	—	—
50	—	0	94	0
81	—	57	—	—

* There were three control groups (1) sperm in untreated sea water, (2) sperm in sea water plus inactive catalase, and (3) sperm in sea water plus active catalase. There was no decrease in survival time of groups 2 and 3 as compared to group 1. Control time was taken as that of group 1.

** Inactivated by heating (60–65° C.) for 30 minutes.

TABLE II

REDUCTION IN TOXICITY OF IRRADIATED WATER BY CATALASE EXTRACT

(Cleavage delay, as compared to controls, in percentage)

Time in experimental medium	In irrad.* water	In irrad. water +inactive catalase	In irrad. water +active catalase	In H_2O_2 (1:1,000,000)	In H_2O_2 +active catalase
10	36	—	22	22	0
40	37	—	18	31	0
50	45	—	11	27	0
40	48	46	0	—	—
40	45	48	45**	—	—
20	58	55	0	—	—
90	82	88	11	—	—

* Approximately 160,000 r.

** Sperm introduced immediately after catalase had been added to the irradiated water.

The delay in time of cleavage also was reduced by adding the catalase extract to the irradiated water or to 1 : 1,000,000 hydrogen peroxide (Table II). Apparently the reason the catalase was not 100 per cent effective in all tests was that in such instances sufficient time was not allowed before the sperm were added to the irradiated water. Maximum efficiency was obtained when the catalase had been in the irradiated water (or hydrogen peroxide solution) for approximately an hour before the sperm were added. In the one test with negative results sperm were added to the irradiated water immediately after the catalase had been introduced, and the

sperm were apparently affected before the H_2O_2 had been destroyed. The amount of catalase used was slightly in excess of that required to remove H_2O_2 (1 : 1,000,000) as determined in Warburg manometers. It is possible that in some instances the amount of H_2O_2 produced by the irradiation was in excess of 1 : 1,000,000. Strong concentrations of catalase were avoided as it was found that too much of the extract was toxic to the sperm.

TABLE III

TOXICITY OF IRRADIATED CATALASE EXTRACT

(Compared with toxicity of irradiated water)

(Toxic effects: reduction in survival time and production of cleavage delay)

Irradiation in 1,000 r	% Reduction in survival time		% Cleavage delay	
	Irradiated catalase	Irradiated water	Irradiated catalase	Irradiated water
84	24	10, 5	14	—
112	58	43, 43	20	55
140	74	58, 58	22	—
168	78	62, 62	25	70
196	80	68, 70	30	—
224	83	75, 75	35	80
140	79	—	40	—
140	74	—	30	—
140	74	—	30	—
140	87	55	43	43
140	55 (diluted to 50% with irradiated water)			
100	65	30	40	55
100	65 (diluted to 99% with active catalase)			
140	44 (diluted to 30% with untreated water)			
140	30 (diluted to 30% with active catalase)			

In an earlier investigation (Evans et al., 1942a) it was found that some egg albumin solutions afforded protection against toxicity of irradiated water whereas others did not. In checking over the data it appeared that protection was afforded only when the albumin had been prepared several days previously. Such solutions, on standing, were found to have developed large numbers of bacteria and possessed catalase activity. Fresh albumin solutions had no catalase activity and did not protect the sperm from the toxic action of irradiated water. Fresh albumin did have a protective effect when present with sperm during irradiation (Evans et al., 1942a). The albumin particles apparently competed with the sperm for the limited number of activated water molecules available and thus reduced the amount of injury to the sperm.

Toxicity of irradiated catalase extract

One type of control in the above experiments (Tables I and II) was the addition of inactive catalase to the irradiated water in which case the toxicity was not reduced. This extract had been made inactive by heating at 60–65° C. for 30 minutes.

It had been found [3] that a dose of 140,000 r would completely inactivate this concentration of catalase, so such an irradiated extract was added to heavily irradiated water to determine whether it would reduce the toxicity. The irradiated catalase failed to reduce the toxicity of the irradiated water, and had even a slightly greater effect in reducing the survival time of the sperm. These results are shown in Table III. Addition of active catalase (two experiments) did not remove the toxicity of the

TABLE IV

EFFECTS OF IRRADIATED ALBUMIN SOLUTION

Exp. No.	Material	½ Survival time in min.	Min. cleavage delay
129	(A) Sperm in control sea water.	70	0
	(B) Sperm in irradiated sea water.	20	15
Irradiation = 160,000 r	(C) Sperm in irradiated sea water + albumin later.	20	20
	(D) Sperm in *irradiated albumin solution.*	48	22
	(E) Sperm in control sea water + albumin.	1,005	0
		(% Survival at 30 minutes)	
20	(E) Control water + albumin.	96	0
Irradiation = 224,000 r	(C) Irradiated water + albumin.	59	40
	(D) *Irradiated albumin.*	57	32
19	(E) Control water + albumin.		0
Irradiation = 224,000 r	(C) Irradiated water + albumin.		42
	(D) *Irradiated albumin.*		42
21	(A) Control water.		0
	(B) Irradiated water.		27
Irradiation = 64,000 r	(F) Irradiated water which had contained 1:100 sperm during irradiation.		5
	(G) Irradiated sperm removed from (F). (Direct effect of irradiation.)		85

radiation-destroyed enzyme extract. Although these results were not conclusive it was indicated that all of the toxicity of the irradiated catalase extract was probably not due to hydrogen peroxide, in other words that some other toxic materials were probably formed. A 0.1 per cent solution of egg albumin was irradiated to determine whether it would be made toxic and the results are shown in Table IV. The tentative conclusion was drawn that the albumin present in the water during irradiation had little effect on the production of hydrogen peroxide by the irradiation, but that albumin itself did not contribute to the toxicity of the solution.

Effects of irradiated water and H_2O_2 on ova

Arbacia eggs placed in irradiated water (168,000 r) or in dilute solutions of hydrogen peroxide in sea water exhibited effects similar to those of sperm so treated. These results are shown in Table V. It was found that immersion in irradiated water or in hydrogen peroxide increased the time for first cleavage and decreased the percentage fertilized. It is of interest that the effects were not as pronounced if

the number of eggs in the experimental medium was doubled. This probably indicates that the number of toxic molecules was limited and the eggs were sensitive to even small changes in the concentration of the toxic materials.

Table V

Effects of Irradiated Water and Hydrogen Peroxide on Ova

(Eggs in experimental medium, inseminated with control sperm)

Experiment 22	Control eggs in sea water	Eggs in irrad. water	Eggs in H_2O_2 (1:1,000,000)	Eggs in H_2O_2 (1:100,000)
Cleavage time: (tested after 30 min. in solution.)	42 min.	48 min.	47 min.	
% Fertilization:	100 fert.	73 fert.	100 fert.	55 fert.
Cleavage time: (tested after 90 min. in solution.)	42 min.	49 min.	48 min.	
% Fertilization:	99 fert.	69 fert.	100 fert.	

Experiment 23	*N* no. eggs in control sea water	2*N* no. eggs in control sea water	*N* no. eggs in (1:100,000) H_2O_2	2*N* no. eggs in (1:100,000) H_2O_2	*N* no. eggs in irrad. water	2*N* no. eggs in irrad. water
Cleavage time: (tested after 25 min. in solution)	47 min.	46 min.	135 min.	103 min.	46 min.	45 min.

Experiment 24	2*N* no. eggs in control sea water	*N* no. eggs in (1:1,000,000) H_2O_2	2*N* no. eggs in (1:1,000,000) H_2O_2	*N* no. eggs in irrad. water	2*N* no. eggs in irrad. water
Cleavage time: (tested after 50 min. in solution.)	46 min.	53 min.	45 min.	52 min.	45 min.
% Fertilization:	100 fert.	100 fert.	100 fert.	56 fert.	100 fert.

Discussion

These results are in agreement with the excellent work of Taylor, Thomas, and Brown (1933) and indicate that when test objects are heavily irradiated (around 100,000 r) in water, the possibility of an indirect effect through production of hydrogen peroxide must be taken into account. Conditions of the medium which have been found to affect the amount of hydrogen peroxide remaining in an irradiated solution are (1) the oxygen content, (2) the pH, (3) temperature, (4) radiation intensity (Fricke, 1934a, 1934b, 1935; Risse, 1930), (5) presence of substances which remove the H_2O_2 (Taylor, Thomas, and Brown, 1933), and (6) the amount of energy absorbed (Bonet-Maurey and Frilley, 1944).

Interference of radiation-produced hydrogen peroxide was reduced to a negligible amount in the earlier investigation (Evans et al., 1942a) by using such dilute suspensions of sperm that they were inactivated by doses (from 2,000 r to 10,000 r) too low to produce detectable amounts of hydrogen peroxide. Also, as hydrogen

peroxide in low concentrations acted only after a time, its toxicity was avoided by removing the sperm immediately from the irradiated water. Taylor, Thomas, and Brown (1933) reduced the toxicity by adding substances possessing catalase activity during the irradiation. In the present investigation, results of attempts to reduce the hydrogen peroxide content by presence of catalase extract during irradiation were puzzling in that the medium possessed toxicity, and it was not removed by active catalase. This finding does not necessarily indicate that irradiation of catalase produces toxic by-products, as many other substances were probably present. However, irradiation of water containing living sperm did not result in toxicity of the medium (Experiment 21-F, Table IV). Other data have been obtained [3] which indicate that catalase *in vivo* is not as easily destroyed by radiation as the catalase extract *in vitro*. Still another possibility is that so many activated water molecules transferred their energy to living sperm that only few remained to produce H_2O_2. Presence of 0.1 per cent albumin did not appreciably affect the production of hydrogen peroxide (Table IV), but possible competing action at higher concentrations was not studied. Catalase extract added to dilute sperm suspensions afforded some protection against the effect of the activated water molecule as studied in the earlier investigation (Evans et al., 1942a). This protection during irradiation was apparently a competing action rather than due to removal of hydrogen peroxide as the active enzyme was no more effective than heat-inactivated catalase extract. The effectiveness of a protecting substance has been indicated by a ratio of the "Median Effect Dose" for sperm in water plus substance over that of sperm in sea water alone. If active catalase is designated as A and inactive catalase as B, the ratios were as follows: Experiment 1, $A = 1.12$ and $B = 1.19$; Experiment 2, $A = 1.6$ and $B = 1.53$; Experiment 3, $A = 2.7$ and $B = 2.6$.

In the previous investigation (Evans et al., 1942a) it was concluded that cleavage delay was caused directly by the radiation, whereas fertilizing capacity (and viability) was reduced indirectly through action of an intermediary (activated water molecule). It is interesting to consider that in the present investigation it was demonstrated that, by producing hydrogen peroxide, the activated molecule could indirectly affect cell division as well as cell viability. The direct effect of the radiation on cleavage delay is considered as the loss of some factors from the nucleus which is replaced in time in irradiated eggs, but not in irradiated sperm through metabolic activity (Henshaw, 1940, I–V; Lea, 1946). Recent discussions of radiation inhibition of cell division (Hevesy, 1945; Mitchell, 1943; and Lea, 1946) suggest the possibility that the effect is due to disturbances in nucleic acid and carbohydrate metabolism by means of enzymatic inactivation. It is generally accepted that hydrogen peroxide poisons many enzymes and that the presence of catalase in cells removes the hydrogen peroxide as it is formed in metabolism. The writer has recently investigated the possible relation between radiation destruction of catalase and radiation production of cleavage delay, but the results indicated that the effects were not necessarily correlated.[3] However, one may still consider that, if both H_2O_2 and radiation retard the functioning of systems which regulate cell division rate, their mode of action may be similar (i.e., oxidation) in producing this effect. The question as to how the active agent in irradiated water (hydrogen peroxide) might have exerted its effect of reducing survival time leads one to consider factors which affect longevity of untreated sperm. This subject has been studied recently by Hayashi (1945–1946) who concluded that a factor prolonging

the duration of fertilizing capacity of Arbacia sperm was adsorbed on the cell surface, and subsequently was lost into the surrounding medium. Seminal fluid contained a protein which, by its action on the surface of the sperm, may maintain fertilizing capacity and respiratory rate. Some findings in the present investigation suggest that the specific protein complex on the surface of the sperm is present even when removed from the seminal fluid by washing and centrifuging, and that its destruction can be retarded by non-specific proteins. This is indicated by the observation that sperm, after removal of seminal fluid, could be kept alive and fertile for extended periods of time (even for days) as long as they were closely packed. In sea water the factor was lost and disappeared more rapidly the more dilute the suspension. It may be noted in the earlier report (and in Table IV, Experiment 129 of this report) that addition of a non-specific protein (egg albumin) increased the survival time of sperm in sea water. Two additional experiments will be cited. In one experiment 1 : 1,000 sperm in 0.1 per cent albumin retained their fertilizing capacity 2.6 times as long as the same concentration of sperm in sea water alone. Sperm in sea water plus albumin, in the other case, remained fertile 14 times longer than sperm in sea water without the albumin (this is the same value as obtained in Experiment 129, Table IV). Sperm in water plus catalase extract remained fertile 1.8 times as long as sperm in water alone. This prolonging of viability was not due to the enzyme activity as heated catalase extract had exactly the same effect (80 per cent increase in survival time). It may be of interest to mention in this connection the finding of Saul and Nelson (1935) and Adams and Nelson (1938) that when purified preparations of invertase or tyrosinase are highly diluted they lose activity. If serum albumin, etc., is added during the dilution, or immediately thereafter, this loss in activity is prevented and activity measurements run proportional to the dilution factor. Hayashi (1946) considers that the fall in activity of Arbacia sperm in sea water is due to destruction of a system involving the cell surface, to auto-intoxication and probable depletion of fuel. One type of auto-intoxication could be auto-oxidation (production of H_2O_2). If this is true then the irradiated water may act by removing catalase from the surface which would, in turn, allow accumulation of H_2O_2 formed during respiratory metabolism. Action of the activated molecule could be due to direct oxidation of materials at the cell surface or to destruction of protective catalase.

Summary

Heavily irradiated water (over 100,000 r) was found to have deleterious effects on sperm placed therein. These effects were reduction of survival time and delay in first cleavage when such treated sperm were used to inseminate fertile eggs. The injury became more pronounced the greater the irradiation dose and the longer the sperm remained in the irradiated water.

The chief, if not the only, agent responsible for these effects of irradiated water was hydrogen peroxide. This was shown by chemical test, by similarity of its action with that of hydrogen peroxide, and by removing the toxicity with catalase extract.

The effects of hydrogen peroxide on fertility and on subsequent cleavage time are discussed regarding possible interpretations of similar reactions of the sperm to more direct effects of roentgen radiation.

LITERATURE CITED

Adams, M. H., and J. M. Nelson, 1938. The use of added proteins in the determination of the activity of tyrosinase. *Jour. Am. Chem. Soc.,* **60**: 2472–2474.

Bonet-Maury, Paul, and Marcel Frilley, 1944. La production d'eau oxygénée dans l'irradiée par les rayons X. *Compt. Rend. des Seances de l'Academie des Sciences,* **218**: 400–402.

Evans, T. C., 1942. Effects of irradiated water on Arbacia sperm. *Biol. Bull.,* **83**: 298.

Evans, T. C., and J. C. Slaughter, 1941. Effect of sea water on the radiosensitivity of sperm. *Collecting Net,* **16**: 101.

Evans, T. C., J. C. Slaughter, E. P. Little, and G. Failla, 1942a. Influence of the medium on radiation injury of sperm. *Radiology,* **39**: 663–680.

Fricke, Hugo, 1934a. Reduction of oxygen to hydrogen peroxide by the irradiation of its aqueous solution with X-rays. *Jour. Chem. Physics,* **2**: 556–557.

Fricke, Hugo, 1934b. Chemical-physical foundation of the biological activities of X-rays. *Cold Spring Harbor Symp. Quant. Biol.,* **2**: 241–248.

Fricke, Hugo, 1935. Chemical properties of X-ray activated molecules with special reference to the water molecule. *Cold Spring Harbor Symp. Quant. Biol.,* **3**: 55–65.

Hayashi, Teru, 1945. Dilution medium and survival of the spermatozoa of Arbacia punctulata. I. Effect of the medium of fertilizing power. *Biol. Bull.,* **89**: 162–179.

Hayashi, Teru, 1946. Dilution medium and survival of the spermatozoa of Arbacia punctulata. II. Effect of the medium on respiration. *Biol. Bull.,* **90**: 177–187.

Henshaw, P. S., 1940. Further studies on the action of roentgen rays on the gametes of Arbacia punctulata. Parts I, II, III, IV, V and VI. *Amer. Jour. Roentgenol. and Rad. Therapy,* **43**: 899–933.

Hevesy, G., 1945. On the effect of roentgen rays on cellular division. *Reviews of Mod. Physics,* **17**: 102–111.

Lea, D. E., 1946. *Actions of radiation on living cells.* Cambridge Univ. Press.

Mitchell, J. S., 1943. Metabolic effects of therapeutic doses of X and gamma radiations. *The British Jour. of Radiol.,* **16**: 339–343.

Risse, P., 1930. Die physikalischen Grundlagen der chemischen Wirkungen des Lichts und der Röntgenstrahlen. *Ergebn. d. Physiol.,* **30**: 242–293.

Saul, E. L., and J. M. Nelson, 1935. The influence of proteins on the activity of yeast invertase. *Jour. Biol. Chem.,* **111**: 95–96.

Taylor, C. V., F. O. Thomas, and M. G. Brown, 1933. Studies on Protozoa. IV. Lethal effects of X-radiation of a sterile culture medium for Colpidium campylum. *Physiol. Zool.,* **6**: 467–492.